

THE EFFECT OF THE SPECTRAL COMPOSITION OF LIGHT ON THE QUANTITY OF THE PHOTOSYNTHETIC PIGMENTS AND ON THE PROPORTION OF COMPONENTS

I. HORVÁTH

Botanical Institute of the József Attila University, Szeged

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Introduction

The absorption of light energy is connected first of all with photosynthetic pigments. According to SIDORIN (1950) the absorption of light is carried out, at least 75 per cent, by photosynthetic pigments.

The role of green and yellow components of the photosynthetic pigment complex in the absorption of light is different. In the opinion of many the energy participating in the photosynthesis is immediately absorbed by the green components, the yellow ones figure as light transformers (DUTTON-MANNING, 1941. EMERSON-LEWIS, 1942, 1951. ARNOLD-OPPENHEIMER, 1950. DUYSENS, 1952. WHITTINGHAM, 1957. TEALE-WEBER, 1957. ALLEN et al. 1961 TAGAWA et al. 1963. FRENCH, 1964), resp. as protective pigments (GRIFFITHS et al. 1955. ANDERSON-FULLER, 1958. ANDERSON-ROBERTSON, 1960). This is proved, e. g., by the fact that the *Chlorella mutants* deficient in carotenoid was fit for life but in the dark, in the light it has perished (CLAES, 1954). According to RABINOWITCH (1951), however, the carotenoids are the primary light acceptors, and that opinion is represented also by JAGENDORF et al. (1958).

It was shown already by GABRIELSEN (1948) that green plants contained much more photosynthetic pigments than those participating actively in photosynthesis. According to VISHNIAC-IRWIN's examinations (1958), in the suspension *Scenedesmus*, exposed in light, only 2 per cent of chlorophyll were marked e. g. by *tricum*. Therefore, the quantity of energy absorbed by photosynthetic pigments may but partly be brought into connection with the photosynthetic productivity. This is referred to also by WIECKOWSKY (1960) who pointed out that in the primary leaves of young bean plants the pigment concentration increased in the course of pigment synthesis is not in connection with the increase of the dry matter content.

Although it may be considered as proved that the quantity of pigments participating in the photosynthetic reaction is but a lesser per cent of the total pigment content, nevertheless, we cannot suppose unambiguously that there is no interconnection between pigment quantity and the productivity of photosynthesis.

According to SESTÁK-CATSKÝ's investigations (1962), a linear connection can be observed between the chlorophyll content referred to the unity of leaf area and the intensity of photosynthesis. At a chlorophyll content of 0.02 mg/square cm per leaf area the productivity of photosynthesis decreases to zero. They render account of similar results also in a later paper (SESTÁK, 1963; SESTÁK-BARTOS, 1963), and even they come to the definite conclusion that under free-earth conditions the productivity of photosynthesis is determined by the chlorophyll content.

We think so that, on the basis of the cited and partly antagonistic statements, the problem may reasonably be raised whether or not there is an interconnection between the quantity of energy absorbed by photosynthetic pigments and the production of organic matter. It is the more reasonable as also we have observed, according to our earlier examinations, a connection between the light absorption of the photosynthetic pigment complex and the quantity of the produced organic matter (HORVÁTH, 1965). The difference in light absorption may have been caused by the quantity of pigments, resp. by the change of the proportion of the single components.

Since the nineteen-fifties the effect of the quality of light and the distribution of spectral energy on the quantity and quality of the photosynthetic pigment complex is examined by more and more scientists. The results can, however, hardly be compared with one another, resp. they are conflicting, supposedly owing to the difficult measurement of the light relations (mainly of the spectral energy distribution).

STOLWIJK (1954) has not found any difference in the absorption of a pigment complex of tomatoes raised in red and blue lights. VOSZKRESZENSZKAJA-GRISINA (1958) has, however, shown on bean plants that the quantity of chlorophyll content increases if influenced by a light of long wavelength. The quality of light influences the quantity of pigment components in different degrees, depending upon the intensity. E. g., the quantity of carotenoids was increased if influenced by red in low light intensity and by blue in a higher light intensity (BRANDT, 1958). KAHNOVICS (1960, 1961 a) has raised plants, under conditioned circumstances, below photoelectric tubes, in lights of different spectral composition, by an identical, physiologically active radiation ($3.1 \cdot 10^4$ erg. cm^{-2} sec^{-1}). He has demonstrated that the quantity of a- and b- chlorophylls had increased as influenced by raising the red and blue wave regions. ALPATOVA (1962 a, b) has examined on young plants the effect of lights of different spectral compositions on the pigments and demonstrated that at photophilous plants the raising of red and yellow wave ranges, — at shade plants however that of blue and violet wave ranges — is favourable for pigment synthesis. According to SZOKOLOVA (1958) the effect of the spectral composition of light on the quantity of chlorophyll depends upon the metabolic character of the plants (she carried out her examinations with autumn, spring, and „walking” wheat species). WAS-SINK-STOLWIJK (1956) see the basis of the spectral sensitivity of plants in the quality of the photosynthetic pigment complex (different ratio of components).

Material and Method

Our examinations were carried out in a light thermostat composed by ourselves (HORVÁTH-KOLTAY, 1963), resp. under free-earth conditions. In the light thermostat photo-electric tubes of differing spectral energy distribution were used. In Table 1 the distribution of spectral energy of the applied photo-electric tubes is demonstrated.

Table 1.

| Photo-electric tube I. | per cent Distribution of energy in the percentage of the energy falling to the wave region between 400—700 nm | | | | | |
|-------------------------------|---|----|----|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | | | | | | |
| F—blue | 13 | 39 | 32 | 6 | 5 | 5 |
| F—green | 0 | 9 | 78 | 9 | 3 | 1 |
| F—red | 0 | 0 | 0 | 1 | 10 | 89 |
| F ₂₉ | 4 | 10 | 25 | 25 | 25 | 11 |

1=violet (400/380—436 nm)

2=blue (436—495 nm)

3=green (495—566 nm)

4=yellow (566—589 nm)

5=orange (589—627 nm)

6=red (627—700/780 nm)

I=wave region

The intensity of illumination at the different photoelectric tubes was identical: 10^{-3} cal. cm^{-2} . $\text{sec}^{-1} \approx 4,10^4$ erg. cm^{-2} . sec^{-1} . The temperature was in a continuous daily rhythm 17—25°C, and the relative vapour content of air changed between 50 and 70 per cent. The CO_2 concentration was constant (0,03 per cent).

At our free-earth experiments the different spectral energy distribution was afforded partly by the seasonal change (LUNDEGARDH, 1954), partly we have got the test material from the undergrowth of different plant substances (acacia, oak, maple-woods). At our free-earth experiments was, of course, also the intensity of illumination different.

The photosynthetic pigment was extracted from the 200—500 mg fresh matter by the method of KOSKI (1950). The quantity of green and yellow pigments was determined after paperchromatographic (WHATMAN 1) separation according to SZAPOZSNYIKOV et al. (1955). The measurement was carried out by JURÁNYI—KOVÁCS's extincio-meter, resp. by „Unicam” absorptio-meter, in a one cm cuvette.

At calculation of the quantity of pigments separated by the paper-chromatographic method the molar extinction of the chlorophylls a and b was considered as a basis, being according to SMITH—BENITEZ (1955) 91 000 in ether, at chlorophyll a, and 48 000 at chlorophyll b, at one cm layer thickness.

The quantitative conversion of carotenoids was calculated according to GOODWIN (1952) so that the extinction of a one percent carotenoid solution was considered, at one cm layer thickness, as 2300 (α -carotene 2940, β -carotin 2150).

The result of pigment examinations was appreciated by Chi²-test, i. e., variancia analyzes and t-test).

Description of results:

Under free-earth conditions we analysed the photosynthetic pigment complex of eight different plants – three cereals, three *papilionaceae*, two underwoods – in March, April, and May of 1961.

The light absorption curve produced on the basis of extinction values of the photosynthetic pigment complex of the examined plants, evaluated by χ^2 test, differed from each other (HORVÁTH, -V. Fehér, 1963, 1964). The difference equally existed at examination of different plants in the same time or of identical plants in different times.

The identity or difference of values was evaluated on the basis of the examination on April 28, 1961 (size of area between the light absorption curve of the photosynthetic pigment complex and the horizontal axis) concerning the wave range between 410–710 nm it was evaluated as a unity and also dissolved into wave areas, with variancia analysis and t-test (Table 2).

Table 2.

square cm

| Plant | Wave region (nm) | | | | | Total |
|-------------------------------|------------------|-------------|-------------|-------------|-------------|-------|
| | 410— 450 | 450— 510 | 510— 570 | 570— 650 | 650— 710 | |
| <i>Secale cereale</i> | 25,2 | 25,8 | 22,4 | 56,5 | 12,0 | 149,9 |
| <i>Triticum aestivum</i> | 26,5 | 26,8 | 22,7 | 60,1 | 16,9 | 153,0 |
| <i>Hordeum vulgare</i> | 25,7 | 24,3 | 17,8 | 49,1 | 10,5 | 127,4 |
| <i>Medicago sativa</i> | 25,3 | 24,7 | 19,3 | 52,0 | 10,9 | 132,2 |
| <i>Trifolium repens</i> | 25,9 | 24,8 | 18,7 | 51,9 | 10,4 | 131,7 |
| <i>Onobrychis viciaefolia</i> | 25,7 | 24,8 | 18,8 | 51,4 | 11,5 | 132,2 |
| <i>Fragaria vesca</i> | 26,5 | 23,5 | 15,3 | 46,3 | 10,6 | 122,2 |
| <i>Glechoma hederacea</i> | 28,5 | 23,4 | 15,7 | 46,3 | 10,0 | 221,2 |

From the evaluation of sections the conclusion may be drawn concerning the connection between the change and the yellow or green pigments.

According to Table 2, the light absorptions of the pigment complex of different plants differ in value, as well. The areas, namely, between the light-absorption curves of the pigment complex and the horizontal axis change between 121,2–153,0 sq. cm, which means a significant difference on a 5 per cent level.

The degree of difference, however, is heterogeneous in every wave range. In the wave area between 450–510 nm, where the light absorption is in connection first of all with yellow pigments, the two extreme values are 23,4, resp. 26,8 sq. cm, meaning a difference but on a 10 per cent level. In the wave area between 570–650 nm, however, which is the absorption maximum of green pigments, the two extremes are 46,3, resp. 60,1 sq. cm, meaning a significant difference even on a 0,1 p. c. level.

It is characteristic of the change of the light absorption of the photosynthetic pigment complex in the function of time that it is generally of increasing tendency

in the period of spring. Thus in the average of eight plants examined in March the area below the curve which is peculiar to the light absorption is 127,3 sq. cm, in April 132,7 sq. cm, in May 134,3 sq. cm.

The quantity of the single pigment components was determined after having been separated with paper-chromatographic method. In Table 3 the total quantity of photosynthetic pigments of the examined eight plants is given, in gamma/g concerning the fresh weight.

Table 3.

| Plant | gamma/g | | |
|-------------------------------|---------|-------|---------|
| | 1—March | 3—May | 2—April |
| <i>Secale cereale</i> | 1169 | 1639 | 1642 |
| <i>Triticum aestivum</i> | 1478 | 1557 | 1487 |
| <i>Hordeum vulgare</i> | 1066 | 1430 | 1439 |
| <i>Medicago sativa</i> | 1242 | 1558 | 1286 |
| <i>Trifolium repens</i> | 1030 | 1398 | 1368 |
| <i>Onobrichis viciaefolia</i> | 1526 | 1148 | 1099 |
| <i>Fragaria vesca</i> | 1219 | 829 | 1126 |
| <i>Glechoma hederacea</i> | 1696 | 1022 | 1638 |

Similarly to the light absorption of pigment complex, in the season of spring also the total pigment content increases. E. g., the increase at rye touches the 40 per cent.

The quantity of pigment components changes, anyhow, not proportionately in the function of time. In the average of the eight plants this change has been summarized in Table 4 (gamma/g fresh weight).

Table 4.

| Date of examination | gamma/g | | |
|---------------------|---------------|---------------|------------------|
| | Chlorophyll a | Chlorophyll b | yellow pigments: |
| March 30 | 672 | 435 | 195 |
| April 28 | 852 | 278 | 191 |
| May 28 | 761 | 421 | 202 |

It can be seen from the Table that first of all the quantity of green pigments change, that of the yellow components but in a low degree. Furthermore, we can see that the quantitative change of the chlorophylls a and b is contrasted.

The change is more evident from the percentage of the participation of pigment components. In Table 5, on the basis of the examinations carried out in March, April, and May, the percentage of the participation of the chlorophylls

Table 5.

| Plant | per cent | | | | | | | | | | | |
|----------------------|-------------|------|-------|------|-------------|------|-------|------|-------------|------|-------|------|
| | March | | | | May | | | | April | | | |
| | Chlorophyll | | | I | Chlorophyll | | | I | Chlorophyll | | | I |
| | a | b | Total | | a | b | Total | | a | b | Total | |
| Corn | 47,0 | 39,3 | 86,3 | 13,7 | 64,0 | 22,0 | 86,0 | 14,0 | 53,6 | 32,0 | 85,6 | 14,4 |
| <i>Papilionaceae</i> | 52,3 | 32,6 | 85,9 | 14,1 | 67,0 | 19,6 | 86,6 | 13,4 | 55,0 | 31,3 | 86,3 | 13,7 |
| Shade-plant | 56,5 | 26,0 | 82,5 | 17,5 | 62,0 | 22,5 | 84,5 | 15,5 | 56,0 | 23,0 | 79,0 | 21,0 |

a and b, as well of yellow pigments of three cereals, three *Papilionaceae*, and two shade-plants is given, as referred to the total pigment content. It is to be noticed concerning the light conditions that the cereals and papilionaceae have obtained an immediate radiation, the shade-plants, however, got it, because of the closed foliage, in a gradually decreasing light which changed in a higher degree concerning the spectral composition.

By reason of Table 5, at the plants that participate in an immediate radiation the proportion of the participation of yellow pigments is in every case nearly 14 per cent, that of green pigments, however, 86 per cent. At both shade-plants the proportion of yellow pigments is higher, and increasing in the function of closing of the foliage. Inside the green pigments, in the season of spring, the percentage of the participation of chlorophyll a was between 47,0 and 67,0, and that of chlorophyll b between 19,6 and 39,3.

Pigment examinations have been carried out on mustard plants *Sinapis alba* L.) of 2–4 weeks, under conditioned circumstances (in light thermostat). On the basis of the summarized result of three experiments it can be ascertained that the light-absorption curves of the pigment complex, valued with χ^2 test, generally differ significantly from one another. In thirteen of the eighteen cases given in our examinations we have got a difference and but in five cases a similarity. A similarity was obtained at plants of different ages, reised in a light of identical spectral composition; a difference of low degree occurred but at plants grown under phototubes F_{29} .

The degree of light absorption is the lowest under „green” and the highest under „blue” phototubes: the area below the curve is 85,6, resp. 125,3 sq. cm, which means a significant difference on a 1 per cent level (Table 6).

It may be concluded from the light absorption of wave region 570–650 nm that the quantity of green pigments is the greatest under blue phototubes (50,2 sq. cm below the curve), and the smallest under the influence of green phototubes (30,3 sq. cm).

The influence of the spectral composition of light on the quantity of yellow pigments is smaller (wave region 450–510 nm): the area below the light-absorption curve is 15,8 sq. cm if influenced by „green” phototubes, and 22,5 sq. cm in case of plants grown under „blue” phototubes.

Table 6.

| Variant | wave region (nm) | | | | | Total: |
|-----------------------------|------------------|------|------|------|------|--------|
| | 410— | 450— | 510— | 570— | 650— | |
| | 450 | 510 | 570 | 650 | 710 | |
| „blue” | 26,3 | 22,5 | 17,4 | 50,2 | 8,9 | 125,3 |
| „green” | 20,4 | 15,8 | 13,3 | 30,3 | 5,8 | 85,6 |
| „orange” (F ₂₉) | 25,1 | 21,8 | 16,1 | 58,6 | 8,5 | 120,1 |
| „red” | 25,3 | 20,8 | 14,9 | 47,7 | 8,6 | 117,3 |

Evaluation of results

It may be ascertained from our free-earth experiments that the light absorption of the photosynthetic pigment complex changes in the function of time, as well, but also in different plants it differs significantly. From the change in the function of time (March, May) a conclusion may be drawn as to the spectral composition of light, as well. That is confirmed also by our experiments carried out under conditioned circumstances. It can be observed that the increase of the proportion of wave ranges that are more absorbed (blue, red) increases the light absorption of the pigment complex. According to the data of Table 6, the increase of light absorption is namely almost parallel with that of proportion of the wave region absorbed in a higher degree by the pigment complex. Anyway, the course of the light-absorption curve of the pigment complex is not changed by the spectral composition of the light.

Furthermore, it can be ascertained that the change of the light absorption of the pigment complex is caused by the change of pigment quantity, resp. by the change of the proportion of components.

On the basis of our free-earth examinations the conclusion may be drawn that the change of absorption of the pigment complex is a result first of all of a change of quantity and proportion of green components. A major change of the green components was concluded by ASCH (1953) in the function of the supply of nutritive matter, by VIDKOVSKAJA-SANGINA (1958) in that of age, by VOSZKRESZENSZKAJA—GRISINA (1958), NESZJAKOVICS—BIBIKAN (1961), KAHNOVICS (1961b) in the function of the intensity of illumination and of the spectral composition of light. Also the quantity of yellow components changes, this change is, however, proportionate to the joint quantitative change of chlorophylls a and b. Consequently the proportion of green and yellow pigments to each other (Table 5) is nearly identical: 6 to 1. The relative stability of the proportion of the green and yellow components is referred to by F. DÁNIEL (1960) and HODORENKO—KLESNIN (1962), as well.

Inside the green component the proportion of chlorophylls a and b changes in a high degree. At cereals, e. g., in the function of time, the participation rate of chlorophyll a is 47–64 per cent, and that of chlorophyll b is 19,6–39,3 per cent.

The comparative stability of the proportion of green and yellow components does not concern, anyhow, the shade-plants.

From the result of our examinations performed under conditioned circumstances we have already emphasized the fact that the spectral composition of light has an effect on the light absorption of pigment complex. The raise of the proportion of the wave region absorbed in a higher degree by the photosynthetic pigment complex increases the degree of light absorption. We have observed also under circumstances conditioned similarly to our free-earth experiments, i. e., on a low energy level, that the effect on green pigments was of higher degree than on yellow ones.

It may also be ascertained that the light absorption of pigment complex, apart from the original and final sections of the ontogenetic cycle, does not change in the function of age in such a degree that it would mean a significant difference, evaluated with χ^2 test.

Summary

On the basis of our examinations carried out under free-earth and conditioned circumstances (light thermostat) it may be ascertained that the spectral composition of light, depending upon a high (free-earth conditions) and a low (light thermostat) energy level, influences the light absorption of the photosynthetic pigment complex partly in a different way.

It may be ascertained that:

- (1) The differing light absorption of the pigment complex of different plants is caused by the change of the pigment quantity and of the single components.
- (2) On a high energy level, except the shade-plants,
 - (2,1) The quantity of pigments generally increases in the season of spring.
 - (2,2) The proportion of green and yellow components is nearly identical (6 to 1), the proportion of chlorophylls a and b is, however, different.
 - (2,3) The change of the light absorption of the pigment complex and of the quantity of pigments taking place in the function of time, under free-earth conditions may only partly be attributed to the light factor, as it is influenced also by the temperature and by the change of other environmental factors, as well.
- (3) On a low energy level:
 - (3,1) The major proportion of participation of the blue and red wave regions increases the light absorption of the pigment complex.
 - (3,2) The effect exerted on the quantity of yellow pigments is smaller than that exerted on the green pigments.
 - (3,3) The light absorption of the pigment complex hardly changes in the function of age (apart from the original and final sections of the ontogenetic cycle).

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